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Trends and approaches in N-Glycosylation engineering in Chinese hamster ovary cell culture

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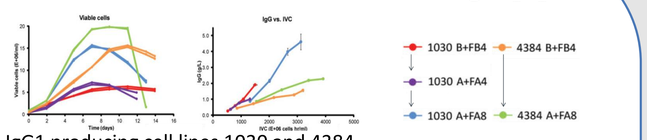
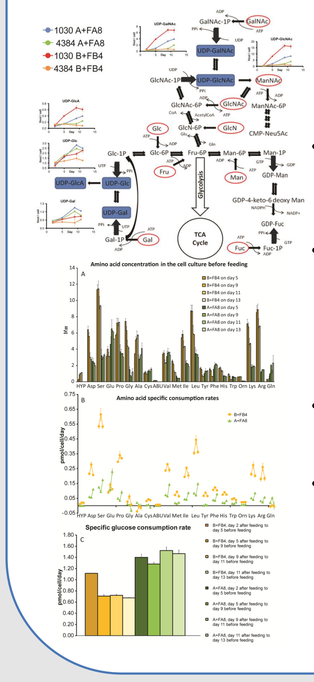
Summary

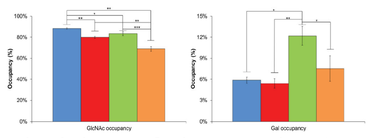
Chinese hamster ovary (CHO) cells have become the preferred expression system for the production of complex recombinant glycoproteins. It has been historically successful in industrial scale-up application and in generating human-like protein glycosylation. N-glycosylation of recombinant proteins, in particular, of those as drug substances, is extremely concerned in drug development and approval, as it will largely affect their stability, efficacy, clearance rate and immunogenicity. Therefore to engineering N-glycosylation of CHO cell-derived recombinant proteins are extremely important. Here, we will summarize a group of recent strategies and approaches and come up with case studies for N-glycosylation engineering in CHO cells and show several examples of relevant study cases from our research: 1) media and feed design, 2) culture process optimization, 3) substrate addition, 4) genetic engineering, 5) omics-based characterization, 6) mathematical modelling.

1. Medium and feed design [1]



- IgG1 producing cell lines 1030 and 4384
- Basal medium A or B; feed medium FA or FB
- Seeding density 0.4 or 0.8 x10E6
- A+FA8 vs.B+FB4: Improved cell growth; higher IVC , higher titer





- The balance of glucose and amino acid concentration in the culture is important for cell growth, IgG titer and N-glycosylation.
- Amino acids with the highest consumption (Ser, Leu) rates correlate with the most abundant amino acids present in the produced IgG, and thus require sufficient availability during culture.
- Higher specific glucose consumption rate is better for cell growth and maturation of glycan.
- Extracellular glucose concentration and its uptake rate were positively correlated with intracellular UDP-Gal availability, which, in turn, resulted in higher galactosylation levels on the complex sugars present on the recombinant product.

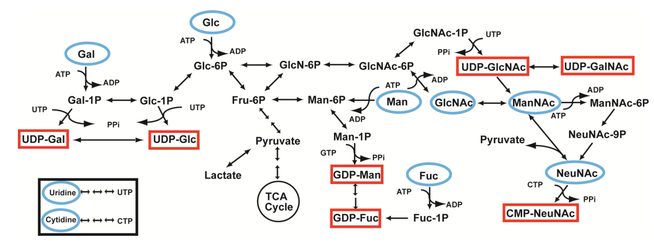
2. Culture process optimization

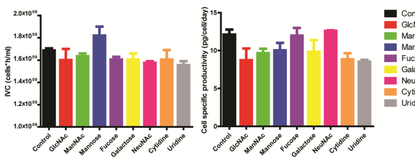
Process parameters	Titer	Critical quality attributes of antibody						
		Acidic isoforms	Basic isoforms	Non-reducible cross-linking	Non-glycosylated form	High mannose	AGOF	Galactosylation
Cultivation duration								
Temperature shift day								
pH (7.2-8.5)								
Osmolality (223-325)								
(100-200) rpm								

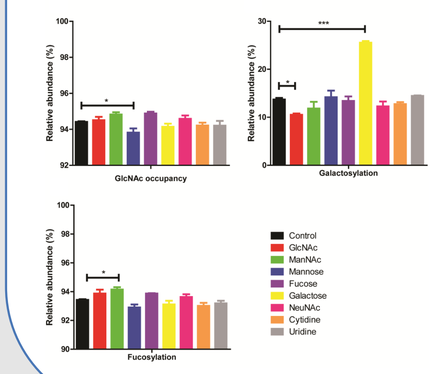
- Strong positive correlation
- Strong negative correlation
- No clear correlation
- Weak positive correlation
- Weak negative correlation

3. Substrate addition [2]

- 8 different substrate additives (glycosylation precursors), including mannose, galactose, fucose, GlcNAc, ManNAc, NeuNAc, uridine, and cytidine were used as feed additives in fed-batch culture ran in triplicates in well-controlled bioreactor systems.

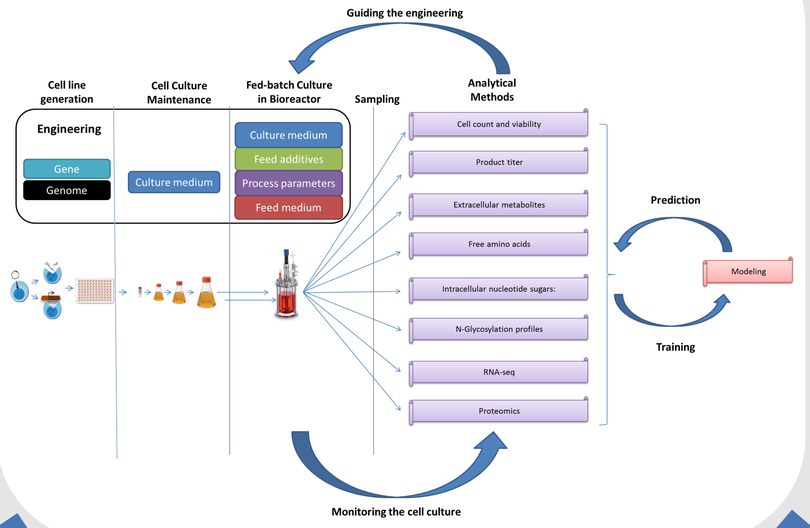






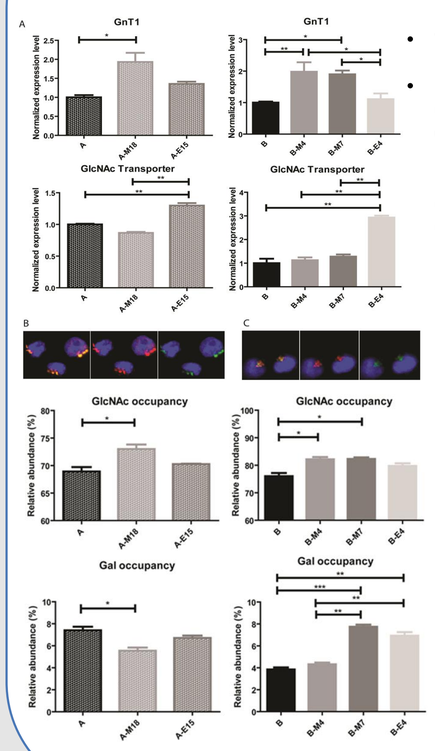
- None of the additives caused statistically significant changes to cell growth and IgG productivity.
- Galactose addition increased galactosylation by 11 %.
- GlcNAc addition reduced galactosylation by 4%.
- Mannose addition slightly reduced GlcNAc occupancy.
- ManNAc addition slightly increased fucosylation.

Work flow in N-glycosylation engineering

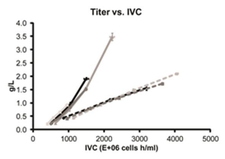


4. Genetic engineering

- Stably overexpress either GnT1 or UDP-GlcNAc transporter in two different IgG producing cell lines A and B.



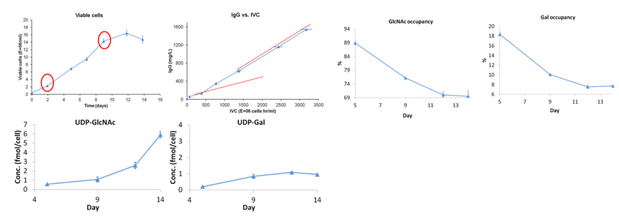
- Western blot quantification.
- Localization confirmation using immunostaining: Overexpressed GnT1-HA and UDP-GlcNAc transporter-FLAG (Red); Golgi Marker (Green); Co-localization (yellow)

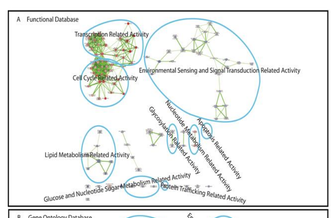


- Overexpression of GnTI: generate less Man5 and higher GlcNAc occupancy.
- Overexpression of UDP-GlcNAc transporter cannot increase GlcNAc occupancy in our cell lines.
- Clone specific effect may affect the average specific productivity of mAb and it is a critical for glycosylation.

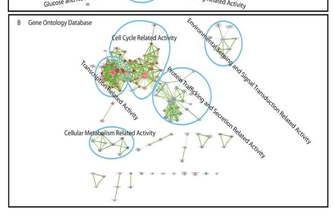
5. Omics-based characterization [3]

•Proteomics analysis on day 2 and day 9 of replicate Fed-batch culture





Day2 up-regulated genes:
•Transcription
•Cell cycle
•Nucleotide metabolism

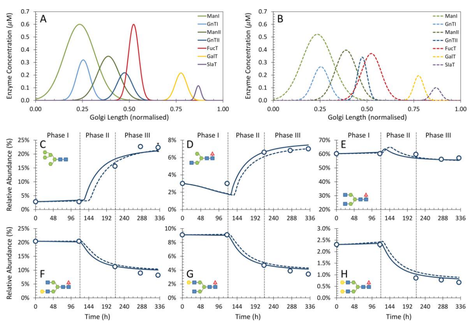


Day 9 up-regulated genes:
•Glucose, lipid, and nucleotide sugar metabolism
•Environmental sensing and signal transduction
•Protein trafficking and secretion
•Glycosylation
•Apoptosis

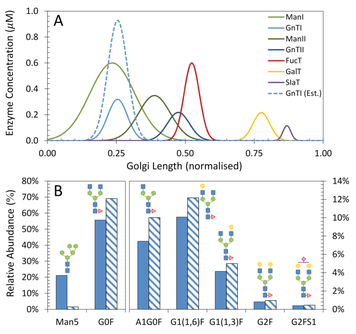
- The overall capabilities of protein secretion machinery from growth phase to stationary phase in the cells gradually exceed the capability of protein glycosylation machinery that is particularly responsible for glycan maturation.

6. Mathematical modelling [4]

- Glycosylation mathematic modeling could aid in cell line selection and engineering during the early stages of bioprocess development



In-silico prediction of dynamic distribution, kinetics and concentration of glycosylation enzymes along the Golgi space



In-silico glyco-engineering: prediction of GnT1 overexpression

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